may be less susceptible to proinflammatory mediators due to diminished oxygen availability. The variation of the enzymatic activity of MRC did not alter the intracellular ATP production since this energy is supported by an increase of anaerobic glycolysis.

## P110

### AGING-ASSOCIATED CHANGES IN ACTIN DYNAMICS IN HUMAN CHONDROCYTES

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**Methods:** The expression of β- and γ-actin as well as vimentin was determined by Western blot analysis of protein extracts from cultured chondrocytes or cartilage obtained from young and old human tissue donors. TGFβ and IL-1β effects on the expression of total beta-actin were determined by Western blot analysis. Age-dependent differences in the ratio of Triton-X 100 to SDS-soluble actin were also analyzed by immunoblotting. Filamentous actin expression in cartilage was determined by confocal microscopy of phalloidin stained thick sections. Differences in cytoskeletal dynamics in response to TGFβ or IL-1β were investigated by fluorescence microscopy and the role of the actin cytoskeleton in TGFβ induction of VEGF or IL-1β induction of iNOS was assessed by real time PCR analysis.
**Results:** Levels of β-actin protein were reduced in chondrocytes from old donors. Stimulation of cultured chondrocytes with TGFβ increased β-actin expression in young as well as in old donors but protein levels remained lower in cells from older donors. In cartilage F-actin expression was increased in the superficial zones of young and old donors and actin was predominantly Triton-X 100-soluble indicating that actin organization in cartilage is characterized by short filaments and monomeric pools rather than submembranous crosslinked networks. Triton-X 100-soluble as well as SDS soluble actin was increased in lesions of OA cartilage when compared to normal appearing areas.
When plated on collagen type II chondrocytes from ypung donors developed stress fibers faster than cells from old donors. However, TGFβ enhanced stress fiber formation in old donors to a greater extent than in young donors while IL-1β prevented formation of stress fibers age-independently.
The microfilament-disrupting drugs cytochalasin B and jasplakinolide altered cell shape and led to disruption of stress fibers in cultured chondrocytes. Both drugs interfered with the TGFβ induction of VEGF mRNA as well as the IL-1β induction of iNOS mRNA.
**Conclusions:** β-actin expression is increased in young versus old donors and cytoskeletal dynamics is age-dependently modulated by TGFβ but not IL-1β. Interestingly, the actin cytoskeleton is involved in the TGFβ induction of VEGF mRNA and the IL-1β induction of iNOS mRNA. Together, these results suggest that differences in chondrocyte microfilament organization can contribute to aging-dependent changes in chondrocyte functions.

## P111

### A TRIAL OF GENE TRANSFER AUGMENTED BY RADIAL SHOCK WAVE FOR RABBIT CHONDROCYTES IN VITRO

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**Aim of study:** The aim of this study was to develop the novel method of gene transfer augmented by radial shock wave.

**Methods:** Chondrocytes were obtained from knee joints of New Zealand white rabbits and cultured on six-wall plate until the cells reached confluency. The cells ware trypsinized and suspended into 5 ml of culture medium, followed by addition of 1 μg of luciferase expression vector (pGV-C2m Toyo-ink, Japan). We used a radial shockwave generator (Swiss DolorClast, EMS, Nyon, Switzerland) in this study. This device generates radial-shaped shock wave, which is different in focal shape and size from conventional focused type shock wave generator. In the shockwave group, shockwaves were exposed to the cell suspension in the different conditions according to such parameters as energy flux density, frequency, shots of exposure. In the control group, the cells were not received any shockwaves. Luciferase activity was measured at 2 days after transfection with a luciferase assay system and evaluated.
**Results:** The transfection efficiency of the shockwave group significantly increased up to 30-fold compared to the control group under the adequate condition of exposure. This method showed the superior enhancing effect to which reported in previous research employing the focused type shockwave generator for gene transfer, without any agents such as microbubble solution. The number of the living cells after the exposure was decreased depending on the number of shots and the energy flux density of shockwaves. As previously reported, the biological effect of shockwave was suggested to involve with these parameters. Thus excessive amounts of exposure may induce the cell disruption which results in a reduction of the transfection efficiency. The frequency of exposure did not concern with both the cell viability and the transfection efficiency in this study.
**Conclusion:** Radial shockwave exposure augmented the transfection efficiency of luciferase expression vector into rabbit chondrocytes in vitro. This method, which is fundamentally safer than the transfection using viral vectors, can be the future option for the clinical gene therapy including osteoarthritis.

## P112

### ALTERATIONS OF CALCIFIED CARTILAGE AND SUBCHONDRAL BONE OBSERVED IN OSTEOARTHRITIC JOINTS: A STEREOLOGICAL STUDY IN TWO STRAINS OF GUINEA PIG

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Subchondral mineralized tissues are believed to play an important role in the pathogenesis of osteoarthritis (OA). Albeit so, very little is known about the relationship existing between calcified cartilage, subchondral cortical and trabecular bone in this disease. The aim of the present study was to investigate the structural properties of these tissues in the primary osteoarthritic joints of two stains of guinea pig.
**Methods:** Two stains of 11-month-old guinea pigs with primary OA [GOHI and Dunkin-Hartley (DH)] were used in this study. The proximal tibias were serially sectioned, and the volume and surface area of the cartilage lesion, total cartilage layer and of the subchondral bone plate (SCP) estimated using unbiased stereological methods. The heights of the hyaline articular cartilage (AC) layer, the calcified cartilage (CC) and SCP were also measured.
The 3-dimensional microstructure of the SCP and of the subchondral cancellous bone was evaluated on micro-CT scans of the femora. To explore site-specific changes in microstructure, we analyzed four regions of interest in the femoral epiphyseal cancellous bone, namely, medial and lateral aspects of the anterior and posterior sites. The total volume of osteophytes was also determined from the 3-dimensional images of the femora.
**Results:** At 11 months of age, structural changes in both strains